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Hydrology induces intraspecific variation in freshwater fish morphology under contemporary and future climate scenarios

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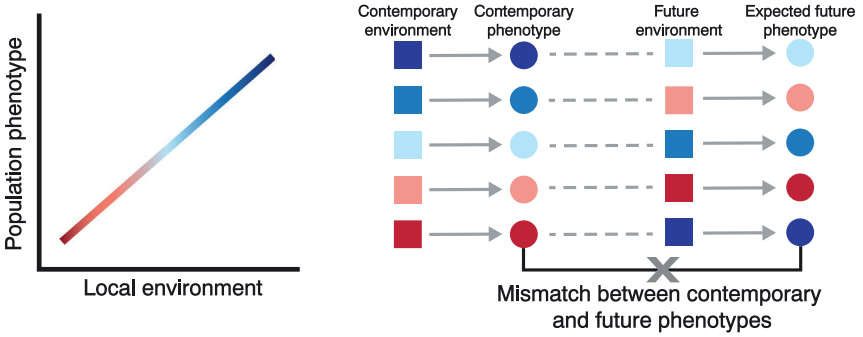
HIGHLIGHTS

- Stream discharge and variability drive intraspecific differences in fish morphology.
- Future morphology (2070–2099) was projected from morphology-hydrology associations.
- Future climate-induced hydrologic changes result in variable projected phenotypes.
- Changes in environmental suitability will vary at the population and species levels.

GRAPHICAL ABSTRACT



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ABSTRACT

Predicting future changes in habitat-associated species traits is an important step in understanding the ecological and evolutionary consequences of environmental change. However, models projecting phenotypic responses to future climate change typically assume populations will respond similarly across the range of a species, while local adaptation and spatial variation in environmental changes are rarely considered. In this study, among-population phenotypic variability was coupled with geographic variation in anticipated hydrologic changes to examine patterns of population-level phenotypic changes expected under future climatic change. To estimate phenotypic responses to watershed hydrology, phenotype-environment associations between body shape and contemporary streamflow were quantified among populations of six species of fishes (Cyprinidae). Future streamflow estimates (2070–2099) were then used to project body shapes within populations, assuming the same phenotype-environment relationships. All species exhibited significant associations between body shape and contemporary streamflow discharge and variability. However, these relationships were not consistent, even among species occupying similar vertical positions in the water column. When these phenotype-environment relationships were projected into future streamflow conditions, populations are not expected to respond uniformly across the species' ranges, and all but one species exhibited projected morphologies outside of the current range of morphological variation. These findings suggest local adaptation and spatial heterogeneity in environmental changes interact to influence variation in the degree of expected phenotypic responses to climate change at both the species and population level.

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1. Introduction

Projecting species' responses to environmental changes is a longstanding yet urgent challenge in ecology. While many studies have examined associations between range shifts, extinctions, and climate change among species, far fewer studies have explored variation in trait-based responses within and among species, particularly in freshwater systems (Crozier et al., 2008; Fuller et al., 2010; Michel et al., 2017). Recent work has demonstrated the potential for adaptive phenotypic and genetic change within species in response to shifting environmental conditions within short time scales (i.e., within decades; Stockwell et al., 2003), including freshwater fishes (Reznick et al., 1997; Franssen, 2011; Crozier and Hutchings, 2014). However, less emphasis has been placed on the importance of responses to spatially varying environmental conditions among populations within species and among closely related species occupying similar types of habitat (Pyron et al., 2007; Haas et al., 2010; but see Franssen et al., 2013a; Hopper et al., 2017; Michel et al., 2017).

Populations occupying habitats across an environmental gradient may be subjected to variable selection pressures, resulting in phenotypic variation across locally adapted populations (Fig. 1A; Williams, 1966). However, most studies projecting the ecological response of species to environmental change assume populations will respond similarly across all environments, with little attention paid to the implications of local phenotype-environment associations (Atkins and Travis, 2010). Moreover, environmental change is expected to vary over geographic space, potentially resulting in high levels of variation in the response of populations across a species range (Fig. 1B; Walther et al., 2002; Knouft and Ficklin, 2017). Because populations are often assumed to respond to environmental changes in a similar manner across the range of species, assessments of the potential impacts of climate change on species traits may lead to spurious conclusions related to the expected displacement of species traits and the capacity of species to achieve their optimal phenotype (Michel et al., 2017). Understanding how variation in the distribution of phenotypes relates to contemporary environmental heterogeneity can therefore provide insight into the potential responses of species to projected environmental changes.

In freshwater systems, projected changes in air temperature and precipitation are expected to affect the timing, magnitude, frequency, duration, and variability of flows that characterize the natural hydrologic regime (Poff et al., 1997; Nijssen et al., 2001; Chien et al., 2013). Streamflow influences inter- and intraspecific variation in morphological traits of freshwater fishes in a predictable manner, where fish in high-flow environments are expected to exhibit streamlined body shapes (e.g. a narrow body depth) to support sustained swimming and minimize drag, and fish in low-flow environments display deep caudal regions and shorter heads to facilitate increased burst swimming ability and maneuverability (Langerhans, 2008; Langerhans and Reznick, 2010). However, the nature of these relationships is not consistent among all species and habitat types (Hendry et al., 2002; McGuigan et al., 2003; Meyers and Belk, 2014), possibly due to the differential effects of streamflow on the body shape of fishes occupying habitats with varying exposure to hydrologic conditions (e.g., high exposure in mid-water habitats vs. low exposure in benthic habitats). Furthermore, streamflow-body shape relationships may also be influenced by the spatial scale at which streamflow is defined. Streamflow is a broad term that has been used to describe discharge ($m^3\ s^{-1}$) velocity ($m\ s^{-1}$), run-off (cm), gradient ($m\ km^{-1}$), or categories such as stream order. These measurements reflect habitat characteristics at different spatial scales, with stream velocity characterizing the local scale microhabitat while streamflow discharge, run-off, gradient, and stream order represent processes occurring at the stream reach-scale (Poff, 1997). Thus, the way in which streamflow is measured may impact the conclusions of the influence of streamflow on body shape (Haas et al., 2015; Beachum et al., 2016).

Morphological differences were examined among populations in six species of freshwater fishes that experience similar gradients of streamflow conditions to investigate how phenotype-environment associations in freshwater fishes can inform expectations of the phenotypic response of fishes to future climatic changes. The first objective was to quantify phenotype-environment associations between body shape and streamflow discharge and variability among species differing in water column position (mid-water vs. benthic). We predict mid-water species will exhibit more streamlined body shapes in high-discharge and high-variability environments, while the body shapes of benthic species will show little association with changes in streamflow variables. The second objective was to use the observed relationships between body shape and streamflow variables to project the phenotypic response of each population under anticipated future hydrologic conditions during the time period 2070–2099. We predict that the combination of differential phenotype-environment relationships among species, among-population trait variability, and geographic variation in anticipated environmental change will produce divergent distributions of phenotypes among species and populations across the landscape in the coming century.

2. Materials and methods

2.1. Specimen collection

Specimens belonging to the Cyprinidae (minnow) family were obtained from the Illinois Natural History Survey (INHS) Fish Collection, where they are preserved in 70% ethanol. Depending on specimen

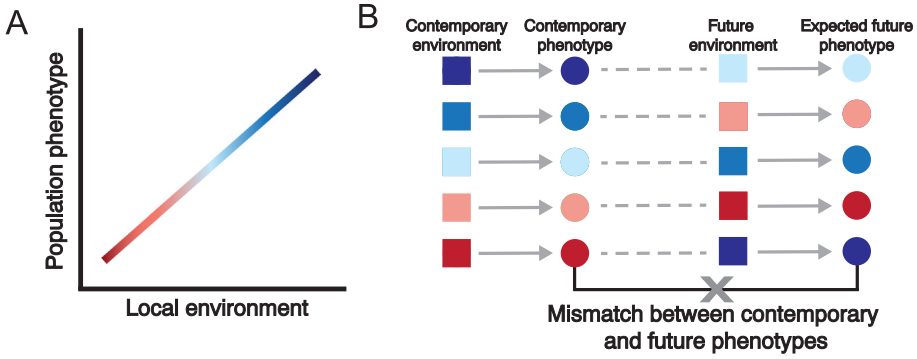

Fig. 1. Due in part to the influence of regional climate and the local physical environment (e.g., soils, elevation, landcover, etc.), local environmental conditions exhibit spatial and temporal variability across the landscape. A, An example of a hypothesized linear relationship between local environments and population phenotypes. B, Under contemporary climates, local phenotypes match the local environment. In the absence of rapid adaptation to future climate change, local phenotypes may no longer reflect the local environmental conditions and deviate from the expected phenotype under future climate change scenarios. The distance between contemporary phenotypes and the expected future phenotypes can differ at the population level.

availability and condition, up to 15 individuals were selected from up to 15 populations of six species, including three mid-water species [*Cyprinella spiloptera* (Spotfin shiner; Cope, 1867); *Luxilus chrysocephalus* (Striped shiner; Rafinesque, 1820); *Notropis atherinoides* (Emerald shiner; Rafinesque, 1818)] and three benthic species [*Notropis buccatus* (Silverjaw minnow; Cope, 1865); *Phenacobius mirabilis* (Suckermouth minnow; Girard, 1856); *Rhinichthys atratulus* (Blacknose dace; Hermann 1804)]. Species were selected based on their availability in the Illinois Natural History Survey fish collection and to span a breadth of habitat preferences and phylogenetic relatedness. Prior to analysis, there was no indication the selected species would demonstrate phenotypic plasticity or local adaptation to streamflow conditions. Water column position for each species (mid-water vs. benthic) was determined based on the mouth position (i.e. terminal vs. subterminal; indicative of foraging location in the water column) and habitat descriptions from existing literature (Supporting information Table S1; Fig. 2). Populations were defined as a group of individuals collected from a single locality during the time period of contemporary streamflow models (1980–2009) and were selected to represent the range of streamflow conditions and geographic extents of each species within the Illinois River, Kaskaskia River, Rock River, and Wabash River basins of Illinois (Fig. 2).

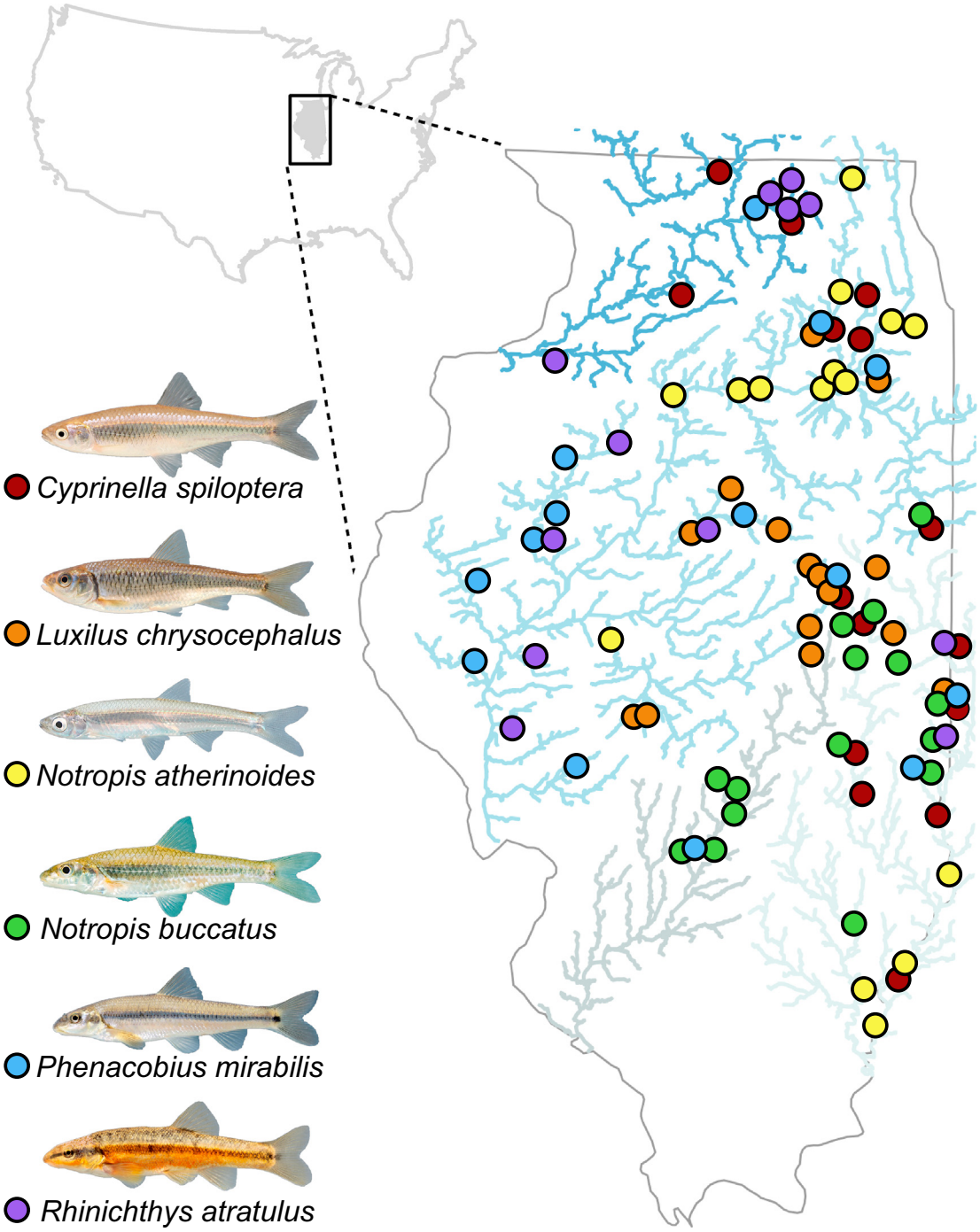

Fig. 2. Location map of populations used in the morphological analyses within the Illinois River, Kaskaskia River, Rock River, and Wabash River basins of Illinois (watersheds are partitioned by color). Points represent the population locations of each of six species: *Cyprinella spiloptera* ($n = 15$; midwater), *Luxilus chrysocephalus* ($n = 15$; midwater), *Notropis atherinoides* ($n = 15$; midwater), *Notropis buccatus* ($n = 15$; benthic), *Phenacobius mirabilis* ($n = 14$; benthic), *Rhinichthys atratulus* ($n = 12$; benthic). Species photographs provided by Matt Thomas.

2.2. Streamflow variables

Contemporary streamflow estimates were derived from a Soil and Water Assessment Tool (SWAT) hydrologic model. SWAT is a landscape-scale hydrologic model that operates on a daily time-step (Arnold et al., 1998). To account for a spatially heterogeneous landscape, SWAT partitions watersheds into several sub-basins, which are then segregated into hydrologic response units (HRUs) based on unique combinations of environmental conditions (e.g., soil, land cover). Outputs from the previously developed SWAT model (Chien et al., 2013) were used to estimate monthly streamflow in 2179 stream sections in Illinois watersheds from 1980 to 2009. From these streamflow estimates, average annual streamflow discharge ($m^3\ s^{-1}$) and the coefficient of variation (CV) of annual streamflow for all stream sections were calculated as described in Chien et al. (2013). Estimates of streamflow discharge and intra-annual streamflow variability were generated for each year from 1980 to 2009. Population-level streamflow estimates for this period were obtained by extracting these values at each population locality (similar to Michel et al., 2017; Table S1). Because populations may exhibit lagged phenotypic responses to streamflow conditions, discharge estimates from the decade preceding the earliest population collection dates were compared with estimates from 1980 to 2009. To generate these discharge data, hydrologic models were updated to estimate monthly streamflows at watershed outlets from 1970 to 1979, and a paired *t*-test comparison indicated that monthly streamflows from 1970 to 1979 did not significantly differ from 1980 to 1989 ($t = 0.77$, $P = 0.443$). Thus, estimates from the hydrologic models represent streamflow conditions relevant to the contemporary phenotypes of the populations.

Future monthly streamflow discharge estimates for the time period 2070–2099 were assembled using SWAT models generated in Chien et al. (2013) with updated climate projections from the fifth phase of the World Climate Research Programme's Coupled Model Intercomparison Project (CMIP5). Twenty global climate models (GCMs) from the CMIP5 multi-model ensemble online archive were assembled under four greenhouse gas concentration trajectories as described in the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (Stocker, 2014; Table S2). These four representative concentration pathways (RCP2.6, RCP4.5, RCP6, and RCP8.5) characterize a succession of radiative forcing trajectories in the year 2100 (Moss et al., 2010). Due to a lack of available data, 13 GCM-RCP combinations were omitted. The total dataset therefore included 67 monthly streamflow simulations for each sub-basin in Illinois watersheds from 2070 to 2099.

Future monthly discharge estimates were used to calculate average annual streamflow discharge ($m^3\ s^{-1}$) and the coefficient of variation (CV) of annual discharge for all stream sections from 2070 to 2099, as described above. To reduce the 67 future streamflow simulations into three representative projections, the average streamflow discharge estimates across all sub-basins were calculated for each streamflow simulation. Based on these calculations, the scenario simulations corresponding to the minimum, median, and maximum average annual streamflow discharge values were selected to characterize three potential future streamflow conditions and corresponded to streamflow projections created using the GCM13-RCP8.5, GCM3-RCP2.6, and GCM7-RCP2.6 combinations, respectively (Table S2). These three models are hereafter referred to as the Minimum, Median, and Maximum future streamflow models.

2.3. Geometric morphometrics

The body shape of each specimen was characterized using landmark-based geometric morphometric methods (Bookstein, 1991; Zelditch et al., 2012). Photographs of the left lateral side of all specimens were taken with a mounted Canon EOS 450D camera. Specimens were not photographed if they exhibited significant bending, deformities, breeding characteristics, or were smaller than 50% of the maximum total length of the species as reported in Page and Burr (2011). Following the exclusion of 18 images due to poor quality, a total of 1081 images remained for analysis. Ten homologous landmarks were digitized on images of each specimen using TpsDig2 software (Rohlf, 2010a). The locations of landmark placement follow Beachum et al. (2016) and were selected to provide coverage of all hypothesized aspects of morphological variation, enabling visual estimates of fin placement (location where the first fin ray connects to the body), body depth (vertical distance from the dorsal fin to the ventral edge of the body), and head size (relative size of region anterior to the operculum). To mitigate effects of bending from specimen preservation, four additional landmarks were placed along the midline of each specimen. Along with the existing landmark at the tip of the snout, landmarks along the midline were used in the 'Unbend specimens' tool in tpsUtil (Rohlf, 2010b); the four added landmarks were subsequently removed from further analysis.

Landmarks of all individuals were aligned by removing the effects of scaling, translation, and rotation with Generalized Procrustes Analysis (GPA; Gower, 1975; Bookstein, 1991) using the 'geomorph' package in R (Adams and Otárola-Castillo, 2013; R Core Team, 2016). GPA retains a measure of centroid size for each specimen, calculated as the square root of summed squared distances of all landmarks from their centroid (Bookstein, 1991). Centroid size serves as a surrogate measure for body size in the analysis. Shape differences among all individuals in each species were summarized with a Principal Components (PC) analysis on the Procrustes-adjusted landmark coordinates using the 'plotTangentSpace' function. Over 80% of the morphological variation in each species was summarized by the first six PC axes, and these axes were therefore retained to summarize variation in body shape in further analyses (Table S3). Due to the focus on intraspecific morphological variation, the GPA and all subsequent analyses were conducted separately for each species.

2.4. Statistical analysis and future morphology projections

Spatial autocorrelation in population-level morphological differentiation was assessed with a Mantel test of pairwise geographic distances and average body shape (PC scores) among populations for each species (Mantel, 1967; as in Ravinet et al., 2013). Spatial distances between populations were calculated as the closest pairwise distances along the stream network using origin-destination (OD) cost distance analysis in ArcGIS 10.4.1 (ESRI, Redlands, CA, USA). After correcting for multiple tests (Bonferroni correction), Mantel tests did not reveal significant correlations between geographic distance between populations and distance along any of the first six PC axes for any species (Table S3).

To assess the relationship between body shape and contemporary streamflow discharge and variability among individuals in each of the six species, linear mixed-effects models were implemented (R package 'lme4'; Bates et al., 2015) with centroid size included as a covariate to account for shape differences resulting from allometry (Loy et al., 1998). The analysis of each PC axis began with (1) a null model comprised of population as a random effect to account for non-independence of individuals collected at the same time and place. Additional models included fixed effects of contemporary estimates of (2) streamflow discharge and (3) intra-annual streamflow variability, as well as the (4) additive and (5) interactive effects between streamflow discharge and variability to determine if the effects of one variable influence the other. All models were then repeated with the inclusion of centroid size as a fixed effect (6–10). Similarly, all models were repeated with the inclusion of random slopes for centroid size; however, random slopes did not improve the performance of models in any species, and the results of these models are not shown. To meet the data assumptions of linear mixed-effects models, centroid size and average streamflow discharge were log-transformed prior to analysis.

The resulting models predicting PC scores for each species were compared using the Akaike Information Criterion corrected for small

sample sizes (AICc), where the best model is indicated with lowest AICc score (Burnham and Anderson, 2002; R package 'AICcmodavg'; Mazerolle, 2017). Models were considered a significant improvement from the null model when ΔAICc exceeds 2.0, and all models within 2.0 ΔAICc were considered to be competitive. When the addition of streamflow variables resulted in significant model improvement, the shape variation associated with the best AICc model for each PC axis was visualized by plotting the landmarks associated with each extreme end of the PC axis (Figs. 3–4). The fit of linear mixed-effect models was assessed with the marginal and conditional R^2, where the marginal R^2 represents the amount of variance explained by the fixed factors alone, and the conditional R^2 considers variance explained by fixed and random factors (Nakagawa and Schielzeth, 2013). Thus, while the marginal R^2 corresponds to variance explained by the fixed effects of streamflow variables and centroid size, the conditional R^2 also accounts for random population-level effects.

The relationships between contemporary morphology and streamflow variables were used to generate the projected phenotype for each individual under future streamflow conditions. Streamflow variables from the Minimum, Median, and Maximum discharge models from the time period 2070–2099 were substituted into the best AICc models relating streamflow variables to body shape. The resulting PC scores summarize the body shape projected for each individual in the Minimum, Median, and Maximum streamflow simulations. The distance between contemporary and future body shape was calculated for each individual as the Euclidean distance between contemporary and future PC scores along all significant PC axes. Analysis of variance (ANOVA) was performed for each species to test for differences in Euclidean distances among populations, hydrologic models, and the interaction between them.

3. Results

3.1. Predictors of morphological variation

Linear mixed-effects models revealed that models predicting body shape were improved from the null model (ΔAICc > 2) with the addition of streamflow variables and centroid size for all species (Tables 1, S4). Centroid size was a model component in either the best AICc model or a competitive model (ΔAICc < 2 from the best model) describing shape changes in PC axes for all species, and was associated with increased body depth, decreased head size, and a superior shift in pectoral fin placement as body size increased (Fig. S2). Allometric changes associated with body depth were especially apparent in mid-water species, while variation in head size and pectoral fin placement were more common in benthic species.

The nature of morphological divergence across streamflow conditions was also variable among species (Figs. 3–4). The shape variation explained by PC axes for mid-water species (*C. spiloptera*, *L. chrysocephalus*, and *N. atherinoides*) was related to streamflow discharge, intra-annual streamflow variability, or the additive or interactive effects between them. Populations of *C. spiloptera* from sub-basins with high streamflow discharge or high streamflow variability contained substantially more streamlined, slender-bodied individuals (PC1), with shifts in the placement of the dorsal, pelvic, and anal fins across a gradient of streamflow variability (PC6). *L. chrysocephalus* individuals inhabiting areas of high streamflow discharge and low variability exhibited head shapes that were less ventrally-angled than conspecifics in low-discharge or high-variability areas (PC4). The dorsal and pectoral fins of *L. chrysocephalus* also shifted anteriorly in sub-basins with high streamflow discharge and high intra-annual

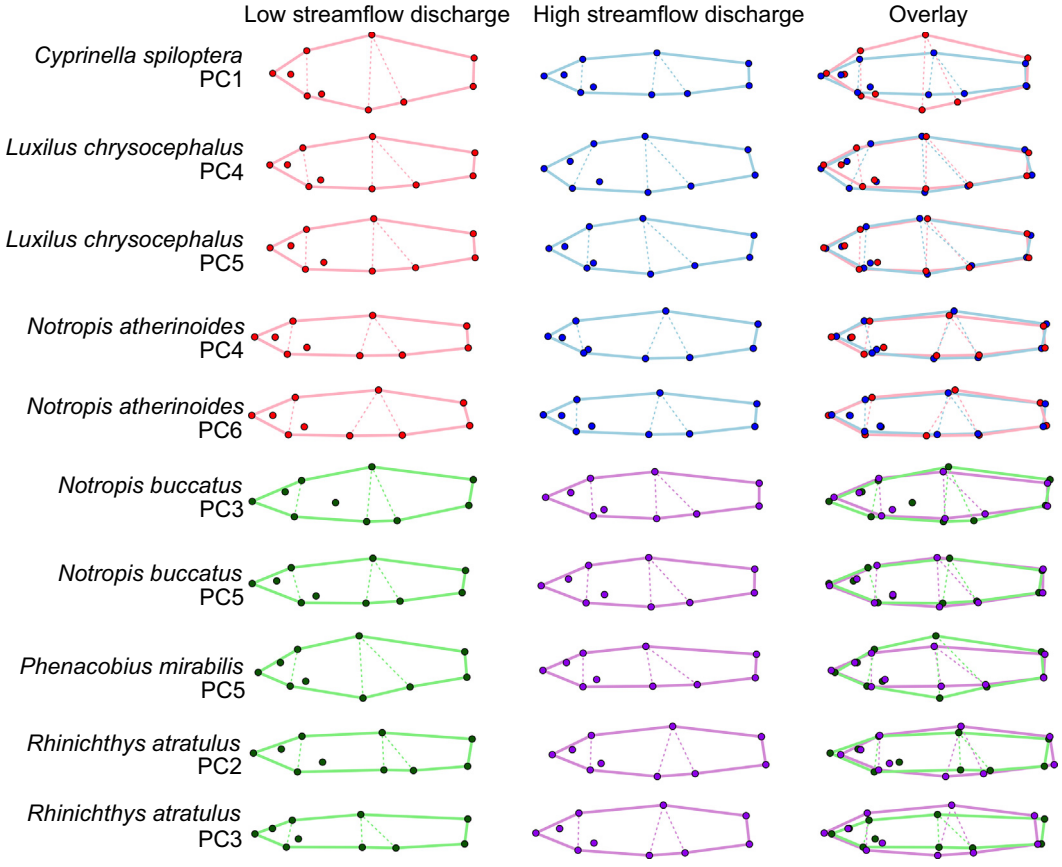

Fig. 3. Changes in body shape associated with landmarks at extreme ends of the PC axes in the best AICc models where the addition of streamflow discharge ($m^3\ s^{-1}$) resulted in significant model improvement (ΔAICc > 2 from the null model). Red-blue landmarks indicate mid-water species, and green-purple landmarks indicate benthic species. To aid in visualization, shape changes are magnified two times.

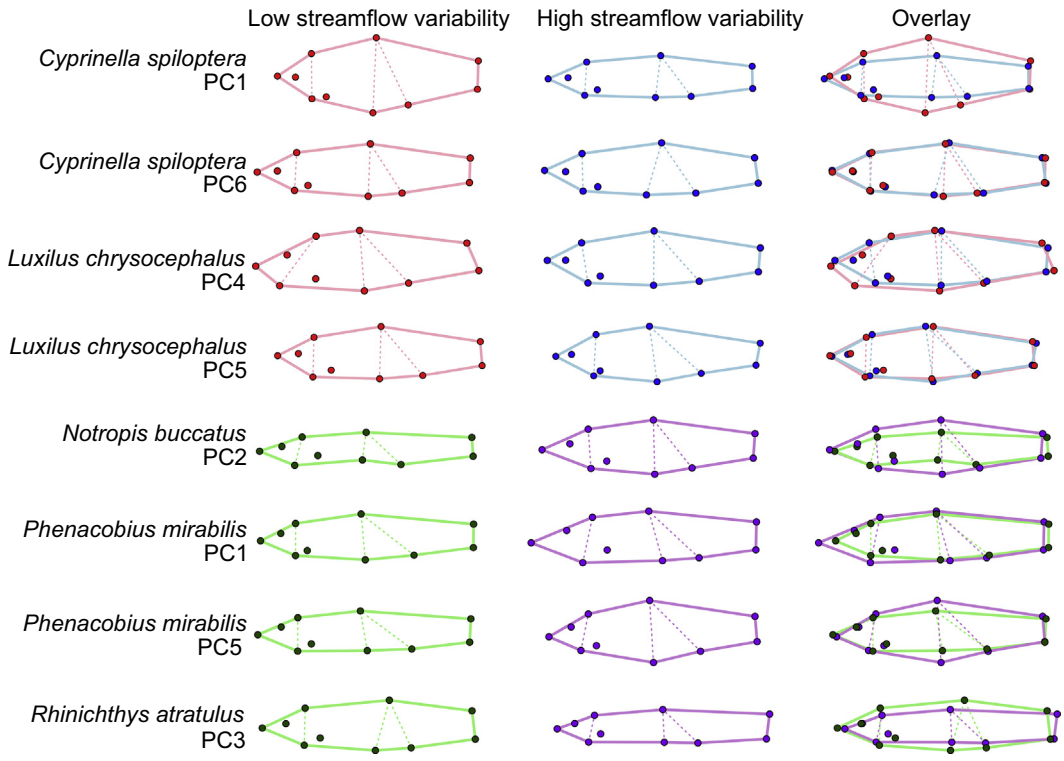

Fig. 4. Changes in body shape associated with landmarks at extreme ends of the PC axes in the best AICc models where the addition of intra-annual streamflow variability (coefficient of variation, CV) resulted in significant model improvement (ΔAICc > 2 from the null model). All PC axes for *N. atherinoides* were <2 ΔAICc from the null model and are therefore not shown. Red-blue landmarks indicate mid-water species, and green-purple landmarks indicate benthic species. To aid in visualization, shape changes are magnified two times.

streamflow variability (PC5). The best AICc models in *N. atherinoides* populations did not show an association between body shape and streamflow variability. In contrast to *C. spiloptera* and *L. chrysocephalus*, populations of *N. atherinoides* from sub-basins of high streamflow discharge contained less streamlined individuals with more ventrally-angled heads than those in low-discharge environments (PC4). Shifts in the dorsal and pelvic fins were also apparent in populations of *N. atherinoides* (PC6).

Benthic species (*N. buccatus*, *P. mirabilis*, and *R. atratulus*) also exhibited unique aspects of morphological divergence that were related to streamflow variables. The dorsal and pectoral fins of *N. buccatus* shifted posteriorly in low-discharge environments, and individuals in populations from low-discharge areas were less streamlined than those in high-discharge areas (PC3, PC5). Additionally, *N. buccatus* was more streamlined and slender-bodied in areas of low intra-annual streamflow variability (PC2). *Phenacobius mirabilis*, on the other hand, exhibited smaller heads at sites with low streamflow variability (PC1), with more streamlined, slender-bodied individuals inhabiting areas of high streamflow discharge and low streamflow variability (PC5). In contrast, populations of *R. atratulus* were more streamlined with anterior shifts of the dorsal fin in low streamflow discharge and high streamflow variability environments (PC2, PC3).

While the body shape for all species was predicted by streamflow variables and body size (Table 1), the nature of the morphology-streamflow relationships and the amount of morphological variation explained varied among species. Marginal R^2, the amount of variability explained solely by fixed factors, was low ($R^2_m < 0.1$) for both significant PC axes in *N. atherinoides* (Table 1). All other species exhibited higher marginal R^2 ($0.1 < R^2_m < 0.3$) for at least one PC axis. With the random effect of population included, the conditional R^2 was often much higher than the marginal R^2, suggesting the importance of population-level effects not attributable to the fixed factors considered in the analysis (Table 1).

3.2. Future body shapes

The Maximum future streamflow model (GCM7-RCP2.6) projects a modest increase in average streamflow discharge across all sub-basins in 2070–2099, while the Median future streamflow model (GCM3-RCP2.6) projects streamflow discharge will be similar to contemporary streamflow discharge and the Minimum future streamflow model (GCM13-RCP8.5) projects a decrease in overall streamflow discharge (Fig. S3). Intra-annual streamflow variability is expected to increase in all three future streamflow models, with the greatest increase in variability seen in the Minimum future streamflow model (Fig. S3). This model reflects the most drastic increases in emissions and is associated with the greatest decreases in streamflow discharge and greatest increases in intra-annual streamflow variability. Correspondingly, the largest differences between contemporary body shapes (PC scores) and future body shapes are expected under the Minimum future streamflow model, while more modest body shape changes are expected to occur under the Median and Maximum future streamflow models (Figs. 5, S4).

While future streamflow conditions allow for estimation of morphological changes for all species, the difference between contemporary and future body shape varies widely depending on the population and PC axis of shape change for each species (Fig. 5). For some species, such as *C. spiloptera*, *L. chrysocephalus*, and *P. mirabilis*, projected future PC scores do not overlap with contemporary PC scores for many populations in the Minimum, Median and Maximum future streamflow models (represented by triangles in Fig. 5). Alternatively, projected future PC scores for populations of *N. atherinoides* always lie within the range of contemporary PC scores, indicating that the body shape of this species under future streamflow scenarios is expected to fall within the current variation already observed by populations in that species. In *N. buccatus* and *R. atratulus*, the PC scores for populations projected by future streamflow models lie within the range of contemporary PC

Table 1
Summary of best models describing shape change summarized by principal components 1–6 for each species. All models are mixed models, with population as a random factor and centroid size as a covariate. All models shown are greater than ΔAICc = 2 from the null model and within ΔAICc = 2 from the lowest AICc model. PC axes in which ΔAICc < 2 from the null model are not shown (full table of model results can be found in Table S4).

Species	PC axis	Model	w_i	cum. w_i	R^2_m	R^2_c
Cyprinella spiloptera	1	Discharge + variability	0.38	0.38	0.233	0.535
		Discharge + variability + size	0.15	0.52	0.236	0.530
	6	Variability + size	0.28	0.28	0.071	0.103
		Variability	0.26	0.54	0.060	0.106
		Discharge + variability + size	0.12	0.65	0.074	0.101
		Discharge * variability + size	0.10	0.76	0.085	0.102
Luxilus chrysocephalus	4	Discharge * variability	0.24	0.24	0.145	0.178
		Discharge * variability + size	0.19	0.43	0.153	0.180
		Discharge + variability	0.12	0.55	0.118	0.176
		Discharge	0.12	0.67	0.096	0.177
		Discharge + variability + size	0.11	0.78	0.128	0.178
		Discharge + size	0.09	0.87	0.106	0.180
	5	Discharge + variability	0.42	0.42	0.098	0.153
		Discharge + variability + size	0.21	0.63	0.102	0.153
Notropis atherinoides	4	Discharge	0.35	0.35	0.069	0.173
		Discharge + variability	0.20	0.54	0.082	0.177
	6	Discharge	0.43	0.43	0.050	0.050
		Discharge + size	0.18	0.61	0.052	0.052
		Discharge + variability	0.16	0.77	0.051	0.051
Notropis buccatus	2	Variability	0.40	0.40	0.128	0.322
	3	Discharge + size	0.46	0.46	0.171	0.334
		Discharge + variability + size	0.31	0.77	0.181	0.328
	5	Discharge	0.39	0.39	0.023	0.023
		Discharge + variability	0.15	0.54	0.024	0.024
Phenacobius mirabilis	1	Variability + size	0.51	0.51	0.221	0.459
		Discharge + variability + size	0.20	0.71	0.226	0.459
	5	Discharge * variability + size	0.44	0.44	0.197	0.306
		Discharge * variability	0.24	0.68	0.179	0.298
Rhinichthys atratulus	2	Discharge	0.33	0.33	0.187	0.276
		Discharge + size	0.20	0.52	0.197	0.271
		Discharge + variability	0.18	0.70	0.200	0.281
	3	Discharge * variability + size	0.29	0.29	0.243	0.375
		Discharge * variability	0.22	0.52	0.225	0.353
		Discharge + variability	0.12	0.64	0.171	0.364
		Discharge + variability + size	0.11	0.75	0.185	0.396

w_i (Akaike weight), likelihood of the model given the data; cum. w_i, cumulative w_i; R^2_m and R^2_c, marginal and conditional R^2 (respectively).

scores on one axis but lie outside of the range of contemporary PC scores on other axes (Fig. 5). In these species, several components of shape change are expected to shift under future climate scenarios, while other shape characteristics may remain the same. Although this projection into novel morphological space makes untested assumptions of consistent phenotype-environment relationships and suitable habitat under novel conditions, these results indicate the potential differential relative responses to changes in environmental conditions among populations.

In all species but *N. atherinoides*, total Euclidian distance between contemporary and future population morphology was greatest under the Minimum future streamflow model, while shape changes projected under Median and Maximum future streamflow models are smaller and similar in magnitude (Figs. S4–S5). Within all species, the degree of shape change is heterogeneous among populations and across space, with significant effects of population on the Euclidean distance between contemporary and future body shapes ($P < 0.001$, Table S5). Projected changes in streamflow are therefore heterogeneous across sub-basins in Illinois, and result in population-specific patterns of projected morphological divergence. The distance between contemporary and future body shapes were also significantly affected by the hydrologic model (i.e., Minimum, Median, and Maximum streamflow simulations), and the interaction between hydrologic model and population in all species but *N. atherinoides*.

4. Discussion

While spatial heterogeneity in climatic changes has been recognized as a potentially important factor when projecting future ecological responses (Walther et al., 2002), most analyses assume a homogenous response of populations across the range of a species (Atkins and Travis, 2010). As a result, species-wide assessments of environmental tolerances and physiological thresholds can misrepresent the ability of populations to persist in future climates, especially in non-migratory species with locally adapted phenotypes (Hällfors et al., 2016). In such species, phenotypic variation and heterogeneous environmental changes can produce varying levels of phenotype-environment mismatching among populations. In this study, fish morphology was variable within species along a gradient of streamflow conditions, highlighting the importance of the streamflow regime as a factor shaping intraspecific patterns of morphology in fishes. Using this phenotype-environment relationship, future changes in streamflow patterns are expected to induce morphological changes in six minnow species. However, the nature of the projected morphological changes is variable among examined species, and the magnitude of projected morphological changes varies among populations in all species. These results demonstrate that intraspecific phenotypic variation and spatially heterogeneous environmental changes lead to population-specific patterns in the expected changes in species traits under changing environmental conditions.

Habitat-induced morphological variation has been found in many species of fishes in a variety of environments (Robinson and Wilson, 1994; Langerhans, 2008). However, few studies have compared the nature of phenotypic variation in populations across multiple species of fishes occupying varying positions in the water column. All species in this study demonstrated divergent morphologies including body depth, fin placement, and head size, yet the PC axes associated with such changes were not necessarily related to streamflow variables. Furthermore, while some species exhibited morphology consistent with a priori expectations of the linkage between streamlining and performance in high-discharge environments, other species exhibited the opposite pattern. Therefore, the role of species habitat preferences in shaping the observed phenotype-environment associations should be considered. For instance, contrary to the prediction that a streamlined body shape is favored in fast-flowing waters, Meyers and Belk (2014) hypothesized that some benthic fishes require sporadic or "burst" swimming abilities (i.e., a reduction in streamlined morphology) that may be beneficial in fast-flowing environments. More robust and less streamlined body shapes may therefore be expected in benthic species occupying these habitats (Meyers and Belk, 2014). While this pattern was found in *R. atratulus*, the other benthic species (*P. mirabilis* and *N. buccatus*) were more streamlined at high streamflow discharge. Furthermore, this hypothesis does not explain why a mid-water species (*N. atherinoides*) exhibited a more robust body shape in high-discharge environments. Additional biotic factors including the presence of predators or competitors (Langerhans and Dewitt, 2004) and availability of prey (Hendry et al., 2002) could also drive patterns of phenotypic divergence in the observed species. These factors do not exist independently of streamflow regime and habitat use, and further studies should test for the presence of additive and interacting selective pressures driving morphological variation in flowing environments.

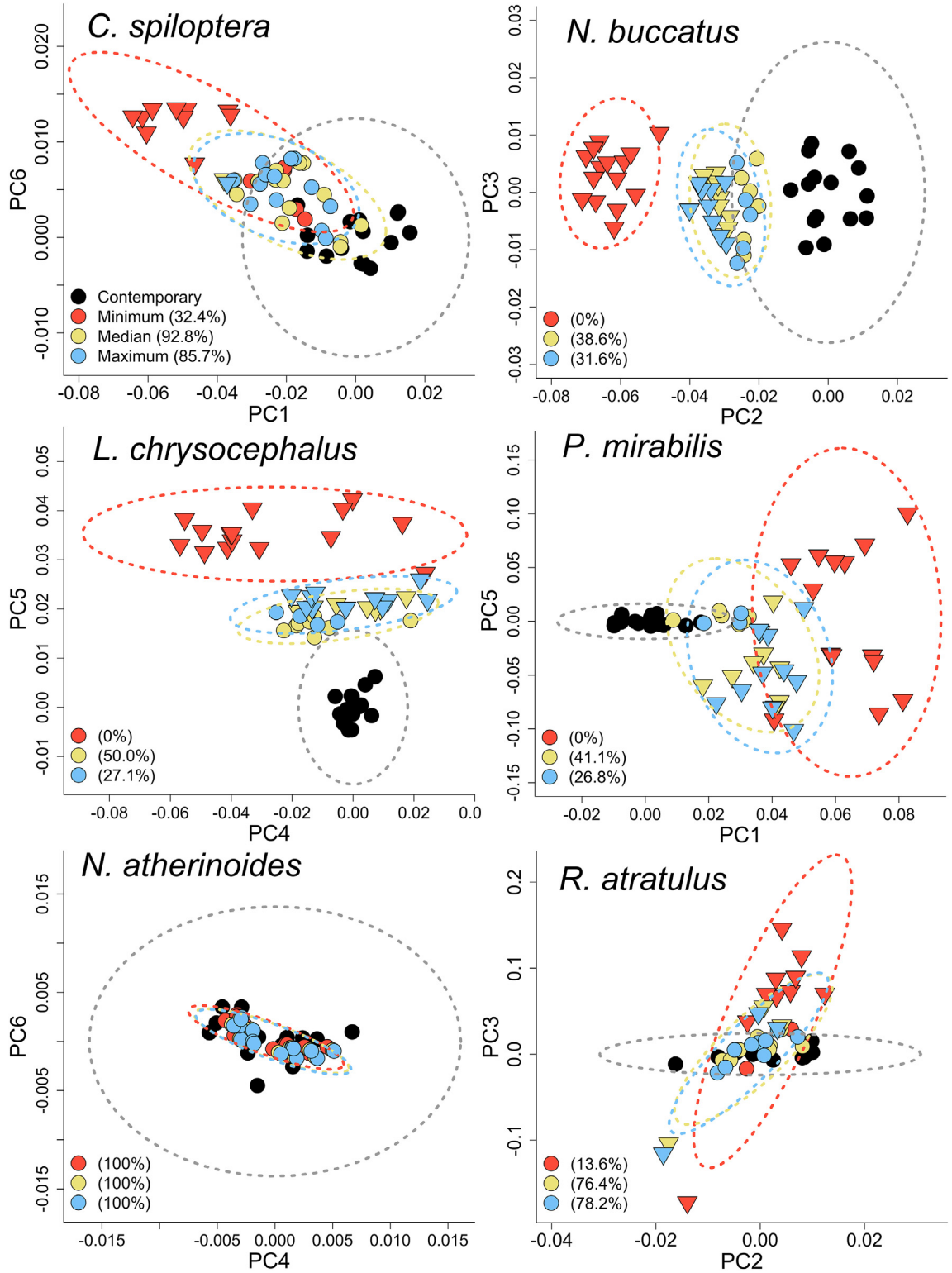

Fig. 5. Principal component (PC) scores for populations of each species along the first two PC axes that were related to streamflow variables. Black points represent the population averages of contemporary body shape, and projected future body shapes are color-coded by the Minimum (orange), Median (yellow), and Maximum (blue) streamflow simulations. Percentages indicate the percent of projected future body shapes within the current range of individual morphological variation, and triangles indicate population averages outside of the current range of individual morphological variation. Ellipses represent 95% confidence intervals.

In addition to habitat preference, the effect of stream size preference and the choice of streamflow measurements are also considered in interpreting the results. For instance, *N. atherinoides* is a mid-water species that occupies the largest streams and highest streamflow discharge of all species in this study; however, this species also exhibited the weakest morphology-streamflow relationships. Because streamflow discharge is equal to the product of stream velocity, depth, and width, high discharge may not necessarily equate to high velocity in large rivers. Streamflow discharge may therefore misrepresent the selective pressures affecting fish morphology in some environments, particularly

if the species specializes in microhabitats within these environments (Senay et al., 2015; Beachum et al., 2016). Thus, although measurements of streamflow discharge and velocity are related, the choice of hydrologic measurement and the spatial scale at which these measurements affect species phenotypes should be considered (Poff, 1997; Pease et al., 2015). Limitations in data availability preclude the inclusion of stream velocity in hydrologic models used in this study. Nonetheless, this study documents the importance of reach-scale hydrology on the body morphology of multiple stream fish species.

These results also document a relationship between intra-annual streamflow variability and the body shape of stream fishes. Although patterns of increased streamlining are expected to reflect higher mobility in variable streamflow environments, species demonstrated a mixture of morphologic responses. Recent studies have acknowledged the importance of including the regime of environmental fluctuations when projecting the ecological and evolutionary responses of species to climate change (Thompson et al., 2013; Vasseur et al., 2014), where increased climatic variability and the presence of stabilizing processes may enhance the variety of responses to extreme climate events (Lloret et al., 2012). Furthermore, because variable conditions are more likely to result in exposure to extreme conditions, the interaction between changes in average conditions and environmental variability should be considered (Walther et al., 2002).

Although all species in the analyses demonstrated morphological responses to a gradient of streamflow discharge and variability, the strength of the relationships between streamflow variables and body shape varied among species. *Notropis atherinoides* inhabits the widest range of streamflow conditions (Fig. S3) but exhibits the weakest relationships with streamflow variables, and projected future morphologies fall within the current range of body shapes. Alternatively, all other species inhabit a smaller range of streamflow conditions and demonstrate stronger morphology-streamflow relationships. In these species, even modest changes in streamflow variables can have a large impact on projected future morphological change, with future hydrologic conditions resulting in body shape projections outside of the current range of morphological variation. Thus, the degree of projected shape change under future streamflow scenarios is not only a reflection of the strength of the relationship between body shape and streamflow variables, but also the amount of projected hydrologic change in the subbasins in which the species resides.

While the approach used in this study provides insights into the potential impacts of climate change on freshwater fishes, there are limitations to the inferences that may be drawn. First, although significant associations between body shape and streamflow variables were found in all species, the relative roles of phenotypic plasticity and microevolutionary responses to natural selection are unknown. Previous studies of morphology-streamflow relationships have documented both genetic and plastic components underlying body shape variation in fishes (Imre et al., 2002; Franssen et al., 2013b), yet determining whether either process can facilitate adequate tracking of changing streamflow conditions over short time scales deserves more attention (but see Michel et al., 2017). A second limitation exists in the extrapolation of phenotype-environment relationships into novel environmental and morphological space. Phenotypic trends are not expected to continue indefinitely as streamflow changes, as species may not be able to achieve expected phenotypes due to functional constraints. Future climate change may therefore result in different phenotype-environment relationships compared to what is currently found in natural gradients (Fukami and Wardle, 2005) and novel environments may no longer be suitable for some populations. However, even when extrapolated beyond the current range of environmental conditions and morphological variation, phenotype-environment associations indicate the direction of anticipated morphological change for each species. Moreover, the differing percentages of populations exhibiting novel morphologies in response to future streamflow scenarios provides a relative estimate of the potential impacts of changes in climate within and among species.

5. Conclusions

This study detected phenotype-environment associations between streamflow regimes and morphological divergence in all species, suggesting morphological adaptation to local streamflow conditions. When this phenotype-environment relationship was projected to estimate the response of species to expected future changes in streamflow regimes, the degree of expected trait change varied among species and among populations within species, demonstrating the potential for both species- and population-level differences in responses to future climatic change. The results of this study demonstrate that populations are not expected to respond to environmental changes in a similar manner across the range of a species and highlight the importance of assessing responses to environmental change at the population level to complement evaluations at the species level. An understanding of spatiotemporal patterns of local adaptation and fine-scale heterogeneity in environmental change is therefore essential for predicting the ecological and evolutionary consequences of rapid environmental change.

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Author contributions

KJA and JHK conceived and planned the study, HC and JHK developed hydrologic models, and KJA collected and analyzed data. KJA, JHK and HC wrote the manuscript.

Data accessibility

Landmark and hydrologic data are available via Zenodo, https://doi.org/10.5281/zenodo.2605428.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.03.292.

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